

DEGRADATION OF BIOMEDICAL WASTE USING MICROORGANISMS ISOLATED FROM VERMICOMPOST AND ITS EFFECT ON GERMINATION AND GROWTH OF PLANT *VIGNA RADIATA*

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ABSTRACT

*The study was intended to analyze biomedical waste degradation with vermicompost amended soil. Vermicompost possess different microorganisms which can advocate for enhanced degradation. In our study, biodegradation of biomedical waste with saline bottles made up of polyethylene was analyzed in 40 days of incubation which exhibits 35.8% using *Bacillus subtilis*, whereas *Pseudomonas aeruginosa* exhibited 9.5% of degradation. The FTIR analysis was carried out to monitor functional group changes occurred due to degradation. A prominent peak of 914.26cm^{-1} exhibited OH-group vibrations. Analysis of plant growth activity in *Vigna radiata* using vermicompost, soil and two types of microorganisms such as *Pseudomonas aeruginosa* and *Bacillus subtilis*. A rapid increase in germination and shoot length with *Bacillus subtilis* which exhibits 80% of germination and $14.5 \pm 1.6\text{cm}$ of shoot length was recorded.*

Key words: *Bacillus subtilis, Pseudomonas aeruginosa, Biodegradation, Vigna radiata, Vermicompost.*

INTRODUCTION

Rapid urbanization leads to the generation of huge amount of municipal solid waste. In other hand due to the booming of medical sector, there is a massive generation of biomedical waste as a result of diagnosis, prevention or treatment of diseases. Disposal of solid waste has become a major problem due to shortage of dumping sites and strict environmental laws. Vermicomposting is a process of accelerated breakdown of organic lignocellulosic wastes by combined action of microorganisms (especially bacteria and fungi) and earthworms in a mesophilic environment [4]. Biodegradation and bioconversion of lignocellulosic wastes has greater importance in production of quality vermicompost rich in beneficial microbes, essential plant nutrients, and humus [7]. The untreated hospital wastes possess serious health hazards to the health care workers, public and air flora on the area source of pharmaceutical products in the environment [6]. Vermiculture biotechnology is an aspect of biotechnology involving the use of earthworms as versatile natural biomedical waste wooden box for processing of various solid, liquid and gaseous wastes into valuable resources [1]. Low density polyethylene is one of the major sources of environmental pollution. Microorganisms that play a role in vermicomposting include bacteria, fungi and actinomycetes [5]. Bacteria metabolize or break down raw organic material and use it as an energy source. Bio degradability is evaluated by weight loss, tensile strength loss, change in percent elongation and changes in polyethylene molecular weight distribution.

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Composting is a natural process of recycling organic material, by the action of biodegradative microorganisms into nutrient rich product known as compost. In the present study an attempt has been made to use Vermitechnology techniques in biomedical waste management by degradation.

MATERIAL AND METHODS

Collection of samples

The biomedical waste samples (saline bottles) were collected from hospital, Pasumalai, Madurai. The soil sample was collected from the hospital dumpsites in Vellakal, Avaniyapuram of Madurai.

Isolation of microorganisms from hospital waste dumped vermicompost

Vermicompost was prepared and collected after 15 days of incubation. One gram of vermicompost was serially diluted and the selected dilutions (10^{-5} , 10^{-6} , 10^{-7} and 10^{-8}) were spread plated onto the sterilized nutrient agar plates and incubated at 37°C for 24 hours. The number of colonies per dilutions was recorded. The colonies with different morphology were identified and it was sub cultured. Four strains (VC1, VC2, VC3 and VC4) were identified and sub cultured to obtain pure culture.

Screening of efficient microorganisms involved in the biodegradation process

In order to test the efficiency of the four selected strain, the selected strains were streaked onto the Mineral Salt Medium (MSM) [8] and incubated. The efficient strains isolated were further characterized morphologically and biochemically.

Morphological and Biochemical characterization:

The two efficient strains (VC3 and VC4) were subjected for morphological characterization performed with gram staining, spore staining and motility. Biochemical characterization of the isolates was performed in accordance to Bergey's manual of determinative Bacteriology.

Biodegradation of biomedical waste using the strains VC3 and VC4

The saline bottles were cut, weighed (initial weight) and washed with sterile distilled water. The strips were amended in MSM aseptically. The control flask was maintained without addition of inoculants and it was taken to compare with the experimental sets carried out separately with VC3 and VC4.

The experimental sets were inoculated with broth cultures separately. All the flasks (Plate: 3) were incubated at 37°C in an incubator for 40 days. The percentage degradation was calculated using the following formula:

$$\text{Percentage (\%) of degradation} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Fourier Transform Infrared Spectroscopic (FTIR) analysis of polyethylene after degradation:

Fourier Transform Infrared spectroscopy (FTIR) is a powerful tool for identifying types of chemical bonds in molecules characteristics of wavelength. FTIR studies were performed to observe functional group modification.

Sample preparation:

The samples from each flask were taken after 40 days of incubation and centrifuged. The pellets were dissolved in small amount of supernatants and then the process of centrifugation is repeated for 3 times. Then the pellets were pooled and air dried and subjected to FTIR analysis. The peaks obtained at different wavelength as functional group modifications were studied.

Morphometric analysis of Mung bean (*Vigna radiata*) by pot culture experiment supplemented with bacterial inoculum and vermicompost

Seeds:

Viable pest free seeds of *Vigna radiata* were purchased commercially from Madurai Agriculture College, Madurai. The seeds were washed and covered with wet cotton cloth and kept overnight.

Soil and Vermicompost:

The rhizosphere soil for the experiment was collected from field, at Pasumalai, Madurai district. The collected soil was autoclaved. The soil was mixed with vermicompost in the ratio 2:1 and filled to 3/4th the capacity of the pots. Then the pots were sown with the sprouted seeds of *Vigna radiata* (5 seeds per pot) and maintained in following basis: **1. Control** – Sterile soil, Vermicompost and seeds. **2. Test-1** – Sterile soil, Vermicompost, seeds and 10ml of *Pseudomonas aeruginosa* (VC3) broth culture. **3. Test-2** – Sterile soil, Vermicompost, seeds and 10ml of *Bacillus subtilis* (VC4) broth culture were carried out for a period of seven days (Table: 1).

Determination of seed germination and growth

Seed germination: The percentage of seed germination was calculated from the each pot with two replicates after seven days from sowing. The percentage of seed germination was calculated using the following formula:

$$\% \text{Germination} = \frac{\text{Total number of germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

Growth: The germinated seeds were further studied for the growth of shoot length in all pots with two replicates and the results have been recorded in cm (Table: 1).

RESULT

Vermicomposting has a tremendous effect on the texture of different substrates. In the present study, vermicompost amended with polyethylene strips were enriched for fifteen days. Isolation and enumeration of bacterial colonies from biomedical waste and vermicompost enriched soil (Plate: 1) by serial dilution was performed. Based on the physiological characterization, the selected colonies were screened for potential isolates using Mineral Salt Medium (Plate: 2). From the results VC3 and VC4 were confirmed as efficient strains that are able to utilize mineral salts as their sole carbon source, which is an important characteristic of a biomedical waste degrading organism. Based on the biochemical characterizations the isolates VC3 and VC4 were identified as *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively using Bergey's manual of determinative bacteriology. In biodegradation assay, the strain VC3 has degraded 0.098 mg of the polyethylene strips, while the strain VC4 exhibited a degradation of 0.179mg of polyethylene (Fig: 1). VC3 and VC4 were showed the rate of degradation of 19.6% and 35.8%, respectively.

The solid based polyethylene studied for the control treated experiment as revealed clear characteristics showing vibrations in CH stretching and bending at 1402cm^{-1} indicating asymmetric covalent bonding and new-functional groups on polyethylene surface. The FTIR results showed that the isolate (*P. aeruginosa*) (Fig: 2) has greatly changed the structure of polyethylene amended with MSM. There was a clear change with the intensity of many groups. The prominent changes were observed in the wavelength at 3313cm^{-1} - 2750cm^{-1} . This change corresponds to change in alkane group (C-H). FTIR indicated a prominent peak with the isolate VC4 (*B. subtilis*) (Fig: 4) suggesting a tremendous change in the structure of polyethylene. The IR peaks obtained with *B. subtilis* showed a prominent peak at a wavelength of 1641.21cm^{-1} which represent the shifting of new bonds in the polyethylene structure which is caused due to biodegradation.

The strains VC3 (*Pseudomonas aeruginosa*) and VC4 (*Bacillus subtilis*) were subjected to pot culture experiment for determining its germination and growth activity of *Vigna radiata*, which revealed 40% and 80% of seed germination, respectively. Further experimental analysis on exposure to vermicompost inoculated with 5% *P. aeruginosa* carried out during 7 days showed decreased (stunted) growth with 0.9 ± 0.8 cm. Similarly, on observation of pot culture of 5% inoculum of *B. subtilis* supplemented along with vermicompost showed 14.5 ± 1.6 cm, a significant difference in growth of shoot length observed in *Vigna radiata* (Table:1).

Plate: 1 Enrichment of vermicompost with biomedical wastes

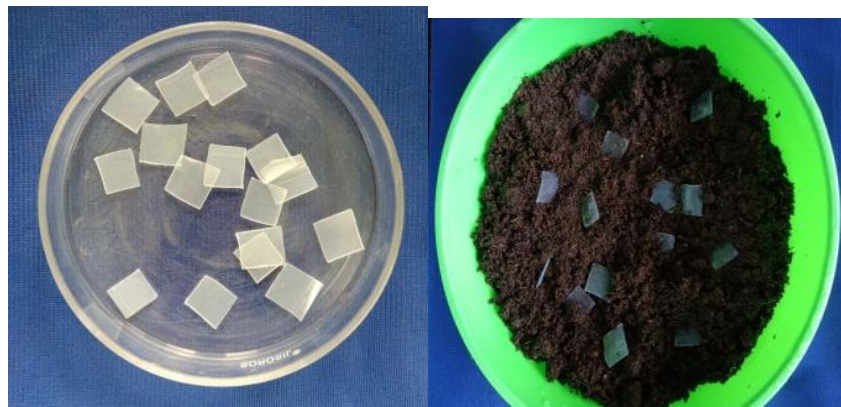


Plate: 2 Screening of potential isolates using Mineral Salt medium

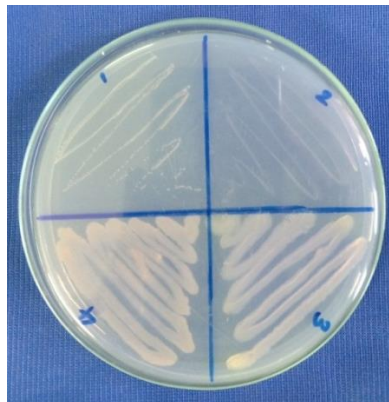


Plate: 3 Biodegradation of biomedical waste using flask culture method

Fig: 1 Degradation of biomedical waste by VC3 (*Pseudomonas aeruginosa*) and VC4 (*Bacillus subtilis*)

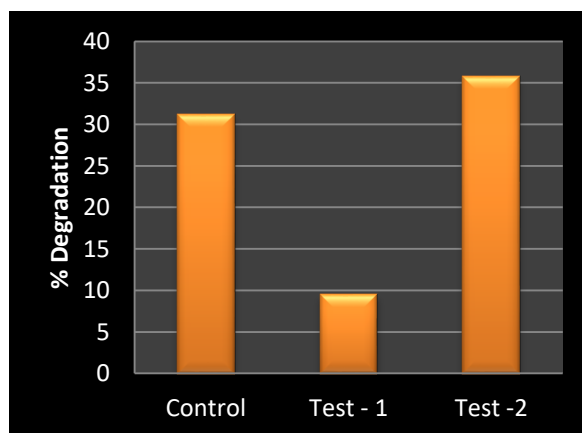


Fig: 2 FTIR analysis of control - Uninoculated MSM containing BMW

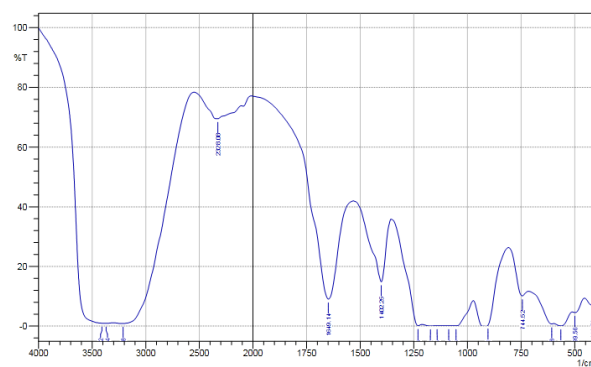


Fig: 3 FTIR analysis of Test-1 MSM containing BMW inoculated with *Pseudomonas aeruginosa*

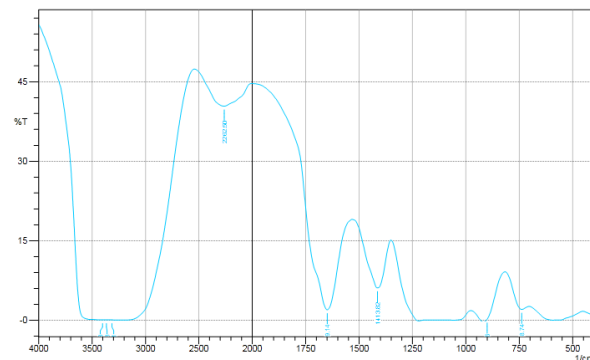


Fig: 4 FTIR analysis of Test-2 - MSM containing BMW inoculated with *Bacillus subtilis*

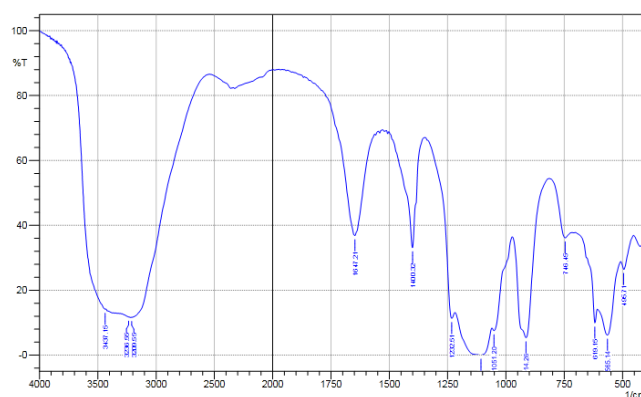


Table: 1 Determination of germination and shoot length of *Vigna radiata* supplemented with and without microorganism

S. No	Experimental samples	No. of planted seeds	No. of germinated seeds	% Germination	Shoot length(cm)
1	Control	5	3	60	13.6±1.2
2	Test – 1	5	2	40	0.9±0.8
3	Test – 2	5	4	80	14.5±1.6

Discussion

Organic manure and beneficial microbes are vital components for improving soil health.

Vermicompost is better and cost-effective technique in degradation of soil waste including biomedical waste. The present study with an aim of adopting vermicomposting to convert the municipal solid waste such as BMW into manure was carried out. Our study coincides with the study of DilipkumarAske (2013). Wherein, he suggested earthworm is beneficial for soil fertility, soil moisture, organic matter from such a point of view, the nutrient contents in biomedical waste treated with earthworm promote degradation at a faster rate and also it induces plant growth.

FTIR results on treatment with *Bacillus subtilis* revealed tremendous change at a short wavelength of 914.26cm^{-1} indicating the vibrations in OH-groups which suggest a rapid biodegradation of polyethylene amended BMW was cleaved by the enzymatic machinery of microorganisms. Our study was in total agreement with the findings of Divyalakshmi and Subhashini (2016). *Vignaradiata* can utilize the compost and the bacterial inoculum for its effective growth, which was substantiated by the significant increase in its percent of germination and length of the shoot length.

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